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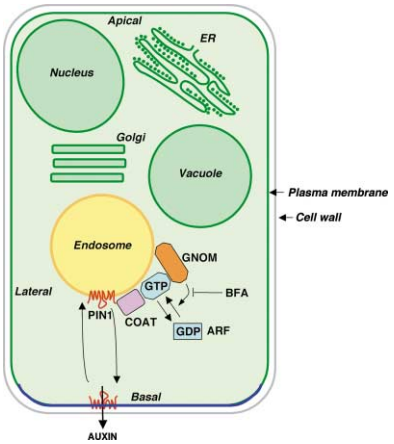
Endosome-Specific Localization and Function of the ARF Activator GNOM

ARF GTPases act at multiple steps of the secretory and vacuolar/lysosomal trafficking pathways, but little is known about the spatial regulation of ARF activation. In this issue of *Cell*, Geldner et al. demonstrate that the *Arabidopsis* ARF activator GNOM localizes to endosomes where it controls the polarized trafficking of the auxin efflux carrier PIN1 to the basal plasma membrane.

ARF (*A*DP-*R*ibosylation *F*actor) proteins are members of the Ras superfamily of small guanine-nucleotide binding proteins. As such, ARFs cycle between a GTP bound, active form and a GDP bound, inactive form. Conversion to the GTP and GDP bound forms is mediated by specific GEFs (*G*uanine-nucleotide *E*xchange *F*actors) and GAPs (*G*TPase *A*ctivating *P*roteins), respectively (Donaldson and Jackson, 2000). A subset of ARFs is referred to as "class I" and comprises two members in humans (ARF1 an ARF3), two in the yeast *Saccharomyces cerevisiae* (ARF1 and ARF2), and six in the plant *Arabidopsis thaliana* (ARF1–ARF6). In all of these organisms, class I ARFs play critical roles in vesicular trafficking at multiple stages of the secretory and lysosomal/vacuolar transport pathways. In particular, these ARFs participate in the formation of transport vesicles from donor compartments and the selection of transmembrane protein cargo for incorporation into the vesicles. They do so by either activating phospholipid-modifying enzymes (i.e., phospholipase D1 and type I phosphatidylinositol 4-phosphate 5-kinase) or recruiting to membranes various cytosolic coat proteins (i.e., COPI, AP-1, AP-3, AP-4, GGA1, GGA2, and GGA3). Each of these proteins has a distinct–albeit, in some cases, overlapping–subcellular distribution. This raises a complex problem of specificity: how can the same ARFs regulate multiple effectors at distinct locations? The answer probably lies with the GEFs and GAPs. Indeed, there are many more ARF-GEFs and GAPs than ARFs, and, among the ones that have been studied, several exhibit rather restricted localizations. Therefore, it is reasonable to hypothesize that particular GEFs and GAPs direct the compartment-specific activation and inactivation, respectively, of class I ARFs. Moreover, the GEFs and GAPs themselves could interact with ARF effectors, thus contributing to the activation or recruitment of effectors at specific intracellular locations.

In mammalian cells, there are five subfamilies of ARF-GEFs, designated Gea/GBF/GNOM, Sec7/BIG, ARNO/cytohesin, EFA6, and ARF-GEP100 (Donaldson and Jackson, 2000). Intriguingly, only two of these families, Gea/GBF/GNOM and Sec7/BIG, exist in *Arabidopsis*, although each has three (GNOM, GNL1, and GNL2) and five members (AtBIG1-5), respectively. Hence it appears that through evolution, plants have expanded the number of members in these two primordial subfamilies, whereas other eukaryotes have evolved new subfamilies. An interesting property of members of the Gea/GBF/GNOM and Sec7/BIG subfamilies is that they are inhibited by the drug brefeldin A (BFA) through stabilization of an ARF•GEF intermediate in the guanine-nucleotide exchange reaction (Peyroche et al., 1999).

Recent studies have begun to test the hypothesis of the compartment-specific function of the mammalian Gea/GBF/GNOM and Sec7/BIG ARF-GEFs. GBF1 localizes mainly to the *cis* aspect of the Golgi complex, whereas BIG1 and BIG2 appear primarily concentrated in the *trans*-Golgi network (TGN) (Zhao et al., 2002). Overexpression of BIG2 stabilizes the association of AP-1 with the TGN, but not that of COPI with the *cis*-Golgi, in the presence of BFA (Shinotsuka et al., 2002b). In addition, overexpression of a dominant-negative BIG2 mutant causes dissociation of AP-1 and GGA1, but not COPI, from membranes (Shinotsuka et al., 2002a). The article by Geldner et al. (2003) in this issue of *Cell* presents more direct evidence for the compartment-specific function of the BFA-sensitive, *Arabidopsis* ARF-GEF GNOM in intracellular protein trafficking. Previous work by these authors demonstrated that mutations in GNOM or treatment with BFA interfere with the polarized secretion of the plant hormone auxin due to mislocalization of its efflux carrier PIN1 (Geldner et al., 2001). Whereas in normal root meristem cells PIN1 shuttles between the basal domain of the plasma membrane and intracellular compartments, in *gnom* mutant or BFA-treated cells, PIN1 accumulates internally. Interestingly, other cellular and developmental processes that require active protein trafficking such as cytokinesis and pollen tube growth are not affected in *gnom* mutant plants. To determine whether GNOM specifically controls PIN1 trafficking, Geldner and colleagues utilized an original approach. They introduced a single amino acid substitution (^{696}M to L) in GNOM to make it resistant to BFA and expressed this GNOM variant in *gnom* mutant plants. Expression of this GNOM variant not only complemented the GNOM mutant phenotype but, more revealingly, also prevented the intracellular accumulation of PIN1 upon treatment with BFA. Moreover, this manipulation rendered both auxin secretion and auxin-mediated growth insensitive to BFA. Since other ARF-GEFs in the same plants remained sensitive to BFA, these observations directly linked GNOM to the polar transport of PIN1 to the basal plasma membrane domain. GNOM labeled with the green fluorescent protein localized to compartments that could be labeled upon short incubations with the fluorescent endocytic tracer FM4-64. In contrast, there was no colocalization with markers for the endoplasmic reticulum, Golgi complex, and TGN, indicating that GNOM is specifically associated with endosomes. In addition, *gnom* mutant cells or wild-type cells treated with BFA exhibited grossly enlarged endosomes. Taken together, these observations indicate that GNOM spe-



Hypothetical Representation of the Function of GNOM

GNOM is peripherally associated with the cytosolic face of endosomes, where it mediates exchange of GTP for GDP on ARF. ARF•GTP in turn recruits to the membrane a putative coat protein that effects concentration of PIN1 at sites of transport vesicle formation. Vesicles carrying PIN1 are then specifically targeted to the basal domain of the plasma membrane. The cell surface PIN1 is subsequently internalized and recycled to endosomes.

cifically mediates the sorting of PIN1 from endosomes to the basal plasma membrane.

The function of GNOM in PIN1 sorting is likely mediated by activation of one or more of the six *Arabidopsis* ARFs (see Figure). The activated ARFs, perhaps in a complex with GNOM, could subsequently recruit to endosomes a protein coat involved in PIN1 sorting. By analogy with mammalian cells, the *Arabidopsis* homologs of AP-1 and AP-4 are particularly interesting candidates to play such a role. In mammalian cells, AP-1 is localized to both the TGN and endosomes, and an AP-1 complex containing an epithelial-specific subunit, μ1B, mediates sorting to the basolateral plasma membrane domain of polarized epithelial cells (Fölsch et al., 1999). Mammalian AP-4 displays a similar subcellular distribution and has also been implicated in basolateral sorting (Simmen et al., 2002). The association of both of these complexes with the TGN and endosomes is dependent on class I ARFs, and BFA inhibits basolateral sorting (Apodaca et al., 1993), implying a role for a BFA-sensitive ARF-GEF in this process. It remains to be determined which of the mammalian ARF-GEFs is responsible for basolateral sorting. In this regard, the methodology of engineering and expressing BFA-resistant variants of sensitive ARF-GEFs utilized by Geldner and colleagues could prove useful to identify the mammalian ARF-GEFs involved in coat recruitment to endosomes and sorting in polarized cells.

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Ubiquitinating a Phosphorylated Cdk Inhibitor on the Blades of the Cdc4 β-Propeller

Substrate binding by the SCF^{Cdc4} ubiquitin ligase is regulated by phosphorylation. In this issue of Cell, Orlicky et al. describe the crystal structure of the Cdc4 subunit bound to a high-affinity substrate phosphopeptide. This structure provides insights into the binding interaction and how a precise mechanism involving multiple regulatory phosphorylations may be mediated by a single binding site.

Initiating DNA replication in fungi and animal cells requires the ubiquitin-dependent destruction of inhibitors of cyclin-dependent kinases (Cdks), a highly regulated event triggered by phosphorylation of those inhibitors on specific sites. These phosphorylated sites or "phosphodegrons" are then recognized by a member of a class of ubiquitination enzymes called SCF ubiquitin ligases through their specific substrate adaptor, known as the F box protein (Feldman et al., 1997; Skowyra et al., 1997). Amazingly, in yeast, the Sic1 Cdk inhibitor is phosphorylated on at least nine sites before it is recognized by the Cdc4 F box adaptor protein. Why so many phosphodegrons for one little adaptor?

SCF ubiquitin ligases are organized around a conserved catalytic core including an E2 ubiquitin conjugating enzyme, a RING finger protein that binds and stimulates the E2, and a cullin, which is an extended protein that serves as a scaffold for the catalytic core (Zheng et al., 2002; Jackson et al., 2000). The cullin in turn binds to a linker protein called Skp1, and Skp1 binds a motif in Cdc4, called an F box. In Cdc4, the substrate binding domain is built on a WD40 domain, which uses repeats of 40 amino acids, each forming four anti-parallel β strands, to assemble the blades of a so-called β-propeller. β-propellers serve in a variety of proteins